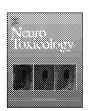


Contents lists available at ScienceDirect

NeuroToxicology



Effects of occupational pesticide exposure on children applying pesticides

Gaafar M. Abdel Rasoul^a, Mahmoud E. Abou Salem^a, Atef A. Mechael^a, Olfat M. Hendy^b, Diane S. Rohlman^{c,*}, Ahmed A. Ismail^a

- ^a Community, Environmental and Occupational Medicine Department, Faculty of Medicine, Menoufia University, Shebin Elkom, Egypt
- ^b Clinical Pathology Department, National Liver Institute, Menoufia University, Shebin Elkom, Egypt
- Center for Research on Occupational and Environmental Toxicology, Oregon Health and Sciences University (OHSU), Portland, OR 97239, USA

ARTICLE INFO

Article history: Received 10 February 2008 Accepted 25 June 2008 Available online 9 July 2008

Keywords: Neurobehavioral tests Adolescents Pesticides AChE Seasonal applicators

ABSTRACT

Nearly 40% of the Egyptian workforce is employed in agriculture. The cotton industry relies on children and adolescents, who work seasonally, to apply pesticides to the cotton crops. Although previous research has examined adult pesticide exposures in this workforce in Egypt, no research has examined the health effects in adolescents. This study attempts to systematically replicate findings examining the impact of organophosphate pesticide (OP) exposure in adults on Arabic speaking children working as applicators. The aim of this study was to examine the impact of pesticide exposure on children and adolescents spraying cotton fields. Male children currently applying pesticides between the ages of 9 and 15 (Younger, n = 30) and 16 and 19 (Older, n = 20) were recruited for the study. They completed a neurobehavioral test battery; personality inventory; work, health, and exposure questionnaires; and medical and neurological screening exams. Blood samples were collected to measure acetylcholinesterase. Children not working in agriculture, matched on age and education, served as controls. Both Younger and Older applicator groups, performed significantly worse than the controls on the majority of neurobehavioral tests controlling for age and years of education. The applicators reported significantly more neurological symptoms than the controls and had lower acetylcholinesterase activity. A dose-effect relationship demonstrated that increased years of exposure to organophosphate pesticides is associated with cognitive deficits. This is one of the several studies demonstrating that functional cognitive effects are positively correlated with increased years of exposure to OP pesticides, though primarily in adult populations, building confidence in the association. Since children around the world are exposed to OP pesticides, these studies suggest that the need to evaluate this potential problem is urgent.

© 2008 Elsevier Inc. All rights reserved.

1. Introduction

Agriculture is the largest employer in Egypt, employing nearly 40% of the Egyptian workforce (Andersen, 2003). The cotton crop is the primary agricultural product and is highly regulated by the Egyptian Ministry of Agriculture (Rizk, 1999). Pesticides, including organophosphorus pesticides (OPs), are used in Egypt on the cotton crop with large quantities being applied relative to other crops (Mansour, 2004). Children and adolescents work seasonally in the cotton fields applying pesticides.

Abbreviations: AChE, acetylcholinesterase; NB, neurobehavioral; PPE, personal protective equipment; WAIS, Wechsler Adult Intelligence Scale; BVRT, Benton Visual Retention Test; BMI, body mass index.

0161-813X/S – see front matter @ 2008 Elsevier Inc. All rights reserved. doi:10.1016/j.neuro.2008.06.009

Adult pesticide applicators and workers who were poisoned by or had high exposures to OP pesticides have reported a broad range of non-specific and neurological symptoms, demonstrated neuropsychological deficits and personality changes, and had acetylcholinesterase (AChE) inhibition associated with exposure to those pesticides. Non-specific symptoms following high exposures have included headache, dizziness, fatigue, weakness, nausea, chest tightness, and difficulty in breathing (Kamel and Hoppin, 2004).

Workers exposed to OP pesticides also demonstrate neurobehavioral deficits on response speed and coordination, sustained attention, visual perception and memory, and complex functioning (Kamel et al., 2003). These studies found that an increasing degree of neurobehavioral deficits were associated with more years working in agriculture and handling pesticides (Rohlman et al., 2007; Roldan-Tapia et al., 2005). An increase in neurological symptoms and neurobehavioral deficits in verbal abstraction, visuomotor speed, problem solving, attention, and memory was

^{*} Corresponding author at: CROET, L606, Oregon Health and Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA. Tel.: +1 503 494 2513; fax: +1 503 494 4278.

E-mail address: rohlmand@ohsu.edu (D.S. Rohlman).

shown in adult pesticide workers in Egypt compared to controls (Farahat et al., 2003).

There has been concern about the impact of pesticides on children and adolescents. Children are considered to be more vulnerable due to the significant anatomical and maturational changes occurring in the brain during developmental periods including adolescence (Andersen, 2003), and also the increasing findings of animal studies indicating neurodevelopment effects of pesticides (Johnson et al., 1998; Moser and Padilla, 1998). Although several studies have examined children who live in agricultural communities or whose parents work in agriculture (Handal et al., 2007; Rohlman et al., 2005), only a few studies have examined adolescents who are currently working in agriculture. Adolescent farm workers in Brazil had motor and attention deficits associated with an exposure index derived by interview (Eckerman et al., 2007). US adolescents working in agriculture had significantly worse performance than adolescents not working in agriculture in attention, memory and visuomotor domains (Rohlman et al., 2001) and increased years working in agriculture and applying pesticides was also associated with neurobehavioral performance deficits in adolescent workers (Rohlman et al., 2007).

This study examined neurobehavioral performance in children seasonally exposed to OP pesticides to test the hypothesis (suggested by Kamel et al., 2003; Rohlman et al., 2007; Roldan-Tapia et al., 2005) that increasing exposure to OP pesticides is associated with progressively larger neurobehavioral deficits, in a population likely to have higher exposure than those previously studied.

2. Methods

2.1. Participants

This study was conducted from June to August 2005, during the pesticide application period to the cotton crop. It was carried out at Shebin Elkom District, Menoufia governorate, Egypt. During this time, the local agricultural office hired approximately 10 children from each village to apply pesticides to the cotton crop under the supervision of adult engineers and agricultural employees. Five villages out of 50 from the Shebin Elkom District were randomly chosen to recruit children working as pesticide applicators as study participants. Male children between the ages of 9 and 18 years currently working as applicators were invited to participate in the cross-sectional study. These children were seasonal workers that spray pesticides in the cotton fields with backpack applicators. There were a total of 56 children working in these 5 villages. Six children declined to participate, yielding a response rate of 89.3%. A control group of children who had never worked in the cotton fields were selected from the friends and relatives of the applicator children. These children lived in the same community as the children who were applicators and attended the same schools. The applicator and control children were divided by age into two groups, Younger (9–15 years of age) and Older (16-18 years of age).

2.2. Pesticide application

The pesticide applications for the cotton crop are highly regulated in Egypt and the Ministry of Agriculture has specific guidelines that are followed throughout the country. Each cotton field in Egypt is subject to four application cycles per season and each application lasts 5–11 days: Cycle (1) *Bacillus thuringiensis* (a natural occurring bacterium that is harmful to insects but not humans); Cycle (2) Pestban (chlorpyrifos) OP application; Cycle (3) Pyrethrins or a less potent carboxylate; and Cycle (4) Dursban (another formulation of chlorpyrifos). There is approximately a week of no spraying between the four application periods and the

total working days for the children were approximately 30 days. This schedule of pest management has been followed rigidly for over 10 years. The typical workday was from 8:00 a.m. to 12:00 p.m. and then from 3:00 p.m. to 7:00 p.m., 6 days per week. Organophosphate pesticides were applied during the second and fourth cycles, from the end of June through the first week of July and during the second week of August.

Agremond backpack sprayers that hold 20 L are worn by the applicators to apply the pesticides. Spraying was carried out by a team of seasonal workers consisting of 7–12 applicators. Applicators are trained by the supervisors to use the backpack sprayers on their first day of work. For the applicators, the typical workday begins at the local agricultural office to receive the backpack sprayers and pesticides. The amount of pesticides applied varies each day depending on the size of the fields to be sprayed and the degree of pest infestation in the fields. Once in the fields, an engineer from the local agricultural office prepares the mixture and loads the backpack sprayers. He also supervises the application process throughout the day. The sprayers are reloaded multiple times during the day until the spraying is completed. Beside the engineers, there are also assistants, and mechanics in the field to oversee the application and maintain the equipment.

Although some personal protective equipment (PPE) is available at the local agricultural office and the applicators have access to it, there are no regulations in Egypt requiring PPE, there is no formal training on its use and it is not commonly used by the applicators. Some wear dust masks and safety glasses to prevent splashes, but this was not the norm in 2005. The main routes of exposure are dermal exposure and inhalation.

2.3. Procedures

Written consent was obtained from the children and their legal guardian. The study was reviewed and accepted by the Medical Ethics committee of the Faculty of Medicine, Menoufia University on July 13, 2005. The children were examined at the end of the workday in a clinic near to their resident villages during the last week of the spraying season.

The children, with the assistance of their parents, completed a questionnaire describing their medical and work history, including information about their exposure to pesticides. A detailed clinical medical examination and a complete neurological examination were administered by specialists. Five milliliters of blood was drawn from all participants and serum AChE was determined according to Weber (1966) using standard kits (Test-combination Boehringer Mannheim GmbH Diagnostica). Serum or plasma AChE was selected because it is a better short-term indicator of cholinesterase inhibition than red blood corpuscles (RBCs) AChE due to its more rapid response to exposure; it is used as an indicator of recent, acute exposure to cholinesterase inhibiting pesticides. Also, because the primary pesticide being applied is chlorpyrifos, which has a preferentially inhibiting effect on serum AChE rather than RBC AChE (Furman, 2006).

Age appropriate versions of the Wechsler Adult Intelligence Scale (WAIS) that were validated in an Arabic speaking population were used to assess neurobehavioral function (Table 1; Abdel-Khalek, 1994). A subset of the tests, Information, Similarities, Arithmetic, and Block Design, had different versions for children (\leq 15 years) and adults (>15 years) participants. The remaining tests in the battery, Digit Span, Benton Visual Retention Test (BVRT), and Trail Making, do not have separate versions for adults and children. Better performance is evaluated by higher scores obtained on tests of Information, Similarities, Arithmetic, Digit Symbol, Block Design, Digit Span, and the Benton Visual Retention Test. In contrast, lower latencies or time to complete Trail Making A and B indicate better

Table 1Name of neurobehavioral test, function, and brief description of the items

Tests	Function	Description
Information	Information and intelligence	30 general information questions for children and 25 for adults
Similarities	Ability to concentrate, abstract	16 pairs of words for children and 12 pairs for adults
	thinking and intelligence	
Arithmetic	Attention and concentration	16 combined oral and written items for children and 10 for adults
Block Design	Spatial relations	Children construct designs using 9 blocks and 10 cards and adults use 16 blocks and 9 cards
Digit Span (Forward, Reverse, Total)	Short-term auditory memory	Sets of three to nine digits, the longest string of digits repeated is the score
Digit Symbol	Perceptual memory speed	90 s to fill in blank squares with correct symbol that matches a digit
BVRT	Short-term visual memory	Participant had 10 s to look at a card, then after 15 s interval is asked to reproduce the drawing
Trail Making (A and B)	Attention, visual conception	Draw a line connecting numbers to numbers (Trail A) and letters to numbers in the correct order (Trail B) ${}^{\circ}$

performance. Examiners were blind to the status of the participant as applicator or control. The Eysenck Personality Questionnaire (EPQ) was used to measure personality. It is formed of 90 questions that assess four aspects of personality; psychoticism, neuroticism, extraversion, and criminality (in addition to the lie scale). A child version was used for participants \leq 15 years old.

2.4. Statistical analysis

SPSS Version 14.0 was used to analyze the data. The *t*-test was used to examine the differences between means, the χ^2 -test was used to examine the differences between percentages, and Pearson's correlation was used to examine the correlation between quantitative variables. Holm's correction of p-values was used to adjust the level of significance (Holm, 1979). Multiple linear regression was used to estimate difference in average performance for control and applicator participants while adjusting for effects of age and education. The difference in performance between these two groups was adjusted to reflect a 13-year-old individual with 6 years of education and a body mass index (BMI) of 21.7 for Younger participants or a 17-year-old individual with 9 years of education and a BMI of 25.1 for Older participants. A second multiple linear regression model was constructed to estimate the partial correlation of NB performance with days worked this season, years worked and AChE, controlling for age, and its interaction with all of these variables.

3. Results

3.1. Demographic characteristics

There were no significant differences on age, years of education or smoking between the applicator and control groups in both the

Younger and Older children. However, for the Older children the body mass index of the control children ($\bar{x} = 26.9$; S.D. = 4.1) was significantly higher than that of the applicator children ($\bar{x} = 23.2 \pm 0.6$) (t = 3.6; p < 0.05).

3.2. Duration of work and AChE

The applicator children reported working as pesticide applicators an average of 5 years (range of 1–9 years). During the current application season they reported working between 3 and 30 days with an average of 20 days. There was no significant difference in days worked during the current season between the Younger applicators ($\bar{x}=19.6$; S.D. = 8.6) and Older applicators ($\bar{x}=21.4$; S.D. = 6.7) (t=0.8; p>0.05). However, the Older participants reported working significantly more years ($\bar{x}=7.2$; S.D. = 1.5) than the Younger participants ($\bar{x}=3.5$; S.D. = 1.6) (t=3.7; p<0.001). Acetylcholinesterase was measured in both the applicator and control groups. A significant lower activity level of AChE was found in the applicator group ($\bar{x}=239.8$; S.D. = 60.0 IU/L) compared to the control group ($\bar{x}=283.1$; S.D. = 61.6 IU/L) (t=3.6; p<0.05).

3.3. Neurobehavioral performance

The applicator groups had significantly impaired neurobehavioral performance (NB) on all of the measures compared to the control groups (Tables 2 and 3). The effect sizes ranged from small (0.2) to large (0.8), using Cohen's classification (1988). After accounting for age and education there was no significant impact of BMI on predicting neurobehavioral performance. The impact of age on modifying NB performance was examined on the tests that were the same for both groups (Digit Span, Digit Symbol, BVRT, Block Design, Trail Making A and B). Age had a significant impact on the BVRT and Digit Span test. For the BVRT, there was a significant

Table 2 Neurobehavioral performance in the Younger (9-15 years) applicator (n = 30) and control (n = 30) groups

	Adjusted mean ^a (S.E.)		B coefficient	95% C.I.	p-Value ^b	Effect size
	Applicator	Control				
Information	20.9 (0.4)	23.4 (0.4)	-2.5	−3.7 to −1.3	< 0.001	0.5
Arithmetic	6.2 (0.6)	8.7 (0.6)	-2.5	−4.2 to −0.9	0.02	0.4
Similarities	18.1 (0.3)	20.8 (0.3)	-2.7	−3.6 to −1.7	< 0.001	1.0
BVRT	4.4 (0.3)	5.9 (0.3)	-1.5	−2.3 to −0.6	0.01	0.6
Digit Span Forward	5.4 (0.2)	6.1 (0.2)	-0.7	−1.3 to −0.2	0.02	0.3
Digit Span Backward	4.7 (0.2)	5.5 (0.2)	-0.9	-1.5 to -0.3	0.03	0.3
Digit Span Total	10.1 (0.4)	11.7 (0.4)	-1.6	-2.7 to -0.5	0.02	0.3
Block Design	33.2 (1.0)	40.0 (1.0)	-6.8	−9.6 to −3.9	< 0.001	0.6
Trail Making A	55.4 (1.0)	48.8 (1.0)	6.6	4.8 to 10.4	< 0.001	1.3
Trail Making B	108.7 (1.4)	104.3 (1.5)	4.5	0.3 to 8.6	0.04	1.3

^a Adjusted to reflect mean performance for a 13-year-old person with 6 years of education and 21.7 BMI.

b Holm's corrected p-value, adjusted for 10 tests.

Table 3 Neurobehavioral performance in the Older (16–18 years) applicator (n = 20) and control (n = 20) groups

	Adjusted mean ^a (S.E.)		B coefficient	95% C.I.	p-Value ^b	Effect size
	Applicator	Control				
Information	16.6 (0.4)	18.4 (0.4)	-1.8	-3.0 to -0.6	0.005	0.1
Arithmetic	5.5 (0.4)	9.1 (0.4)	-3.6	-4.7 to -2.5	< 0.001	0.2
Similarities	15.9 (0.2)	18.9 (0.2)	-3.0	-3.6 to -2.4	< 0.001	0.4
BVRT	4.6 (0.2)	6.3 (0.2)	-1.7	-2.2 to -1.2	< 0.001	0.2
Digit Span Forward	4.0 (0.1)	5.9 (0.1)	-1.9	-2.3 to -1.6	< 0.001	1.0
Digit Span Backward	3.5 (0.2)	5.5 (0.2)	-2.0	−2.5 to −1.5	< 0.001	1.0
Digit Span Total	7.5 (0.3)	11.3 (0.3)	-3.7	−4.5 to −3.0	< 0.001	0.5
Block Design	18.3 (0.7)	26.1 (0.7)	-7.7	−9.6 to −5.8	< 0.001	0.1
Digit Symbol	25.2 (0.8)	33.5 (0.7)	-8.4	-10.5 to -6.2	< 0.001	0.4
Trail Making A	60.4 (0.8)	54.1 (9.8)	6.3	4.1-8.6	< 0.001	0.3
Trail Making B	118.8 (1.2)	111.9 (1.1)	6.8	3.5-10.1	< 0.001	0.1

^a Adjusted to reflect mean performance for a 17-year-old person with 9 years of education and 25.1 BMl.

interaction between exposure and age (p < 0.001), for a participant with 6.6 years of education, BVRT scores of the controls increased 0.3 units for each additional year of age, while decreasing 0.3 units for each year of age among applicators. There was also a significant interaction on the Digit Span test between exposure and age (p = 0.001). For a participant with 6.6 years of education, Digit Span scores of controls increased 0.2, 0.3, and 0.8 units for each additional year of age for Digit Span Forward, Backward, and Total scores, respectively, while decreasing 0.5, 0.4, and 0.6 units for each year of age among applicators for Digit Span Forward, Backward, and Total, respectively.

Both the Younger and Older applicators scored significantly higher on the neuroticism (mean of 10.0 for both Younger and Older, respectively) and extraversion scales (mean of 13.2 for both Younger and Older, respectively) than the control participants (mean of 9.2 for both Younger and Older on the neuroticism scale and mean of 11.9 for both Younger and Older on the extraversion scales).

3.4. Neurological symptoms

The neurological examination revealed an increase in neurological symptoms in the applicator group compared to the control group. The χ^2 -test demonstrated that significantly more participants in the applicator group had blurred vision (28%), dizziness (24%), difficulty in concentration (24%), trouble in remembering (24%), feelings of depression (26%), irritability (22.%), numbness (18%), and fatigue (30.0%), compared to the control participants (8%, 8%, 8%, 6%, 10%, 6%, 2%, and 10%, respectively) (p < 0.05). The

Table 4Correlation (partial) of the neurobehavioral performance with duration of work (days worked in current season and years worked as an applicator) and AChE

Neurobehavioral tests	r				
	Days worked	Years worked	AChE		
Information	-0.6**	-0.1	0.4*		
Arithmetic	-0.7**	-0.1	0.3		
Similarities	-0.5**	-0.4*	0.2		
BVRT	-0.8**	0.2	0.1		
Digit Span Forward	0.3	-0.2	0.4*		
Digit Span Backward	-0.1	-0.2	0.4*		
Digit Span Total	-0.2	0.0	0.4*		
Block Design	-0.3*	-0.6**	0.2		
Digit Symbol	-0.4	0.1	-0.2		
Trail Making A	0.1	0.2	-0.1		
Trail Making B	0.4*	0.5**	0.3*		

p < 0.05, p < 0.001.

same results were obtained when the Younger and Older groups were analyzed separately.

Applicator children who report at least three of the following neurological symptoms, blurred vision, dizziness, headache, difficulty in concentration, trouble in remembering, difficulty in understanding, feeling depressed and irritable, numbness, low back pain, and fatigue, worked significantly more days ($\bar{x}=27.4$; S.D. = 4.1) during the current season than applicator children who report less than three symptoms ($\bar{x}=16.7$; S.D. = 6.8) (t=6.0; p<0.001). Applicator children who report at least three of the previously mentioned neurological symptoms also worked significantly more years applying pesticides ($\bar{x}=6.2$; S.D. = 2.4) than applicator children who report less than three symptoms ($\bar{x}=4.3$; S.D. = 2.1) (t=3.0; p<0.05).

3.5. Correlation of neurobehavioral performance with AChE, duration of work

Partial correlation of NB performance with the days worked this season, years worked as pesticide applicators and AChE was estimated by constructing a regression model controlling for age, and its interaction with days or years worked (Table 4). A significant correlation between the days worked during the current season was found with performance on 6 of the 11 measures. The Information, Arithmetic, Similarities, BVRT, and Block Design subtests had significant negative correlations and Trail Making B had a significant positive correlation (p < 0.05). Years worked as pesticide applicators showed a significant correlation with 3 of the 11 measures, BVRT, Block Design, and Trail Making (p < 0.05). A significant correlation between AChE and 5 of the 11 measures was also found (Information, Digit Span Forward, Backward, and Total, and Trail Making B; p < 0.05).

4. Discussion

This study reveals significant health effects in children who work as pesticide applicators compared to control children. Children who apply pesticides show impaired neurobehavioral performance, report more symptoms, and have lower AChE activity levels than children from the same communities that do not apply pesticides. This study also shows a correlation between days worked during the current season and total years worked as an applicator and the reporting of symptoms and neurobehavioral performance which provides evidence of a dose–response relationship between exposure and both symptoms and neurobehavioral performance. The strong relationship between the exposure to pesticides and decrement in the different functions of NB

b Holm's corrected p-value, adjusted for 11 tests.

performance is also confirmed by the presence of significant correlations between the days worked this season on 6 of the 11 NB tests (Information, Arithmetic, Similarities, BVRT, Block Design, and Trail Making), while the years worked as an applicator had significant correlations with 3 of the 11 NB tests (BVRT, Block Design, and Trail Making B) after controlling for age, and its interaction with the days and years worked. This confirms other research results (e.g. Roldan-Tapia et al., 2005), that also found a significant relationship between the period of exposure and working in applying OP pesticides and performance deficits and increased neurological symptoms.

This is the first study in children to demonstrate a doseresponse relationship between functional cognitive effects, where these functions are positively correlated with increased years of exposure to OP pesticides. These findings confirm previous reports of a relationship between increased years working in agriculture and increased performance deficits found in adult and adolescent workers (Kamel et al., 2003; Rohlman et al., 2007; Roldan-Tapia et al., 2005). The demands of the job require the adolescents to work long days in hot and humid conditions. Although these could contribute to an increased reporting of symptoms of fatigue and irritability, other studies with adult populations report similar findings (Farahat et al., 2003; Stallones and Beseler, 2002). Furthermore, both the applicator and control participants are used to working long hours in these conditions, which make it unlikely that working conditions and not pesticide exposure are the cause of the increased symptoms reporting in the applicator children.

This decrement of neurobehavioral performance, and reporting of more neurological symptoms in the applicator participants, is consistent with a previous study that examined adult pesticide workers in Egypt (Farahat et al., 2003) which found significant deficits in complex visual-motor processing and executive function, verbal abstraction, attention and short-term memory and memory/problem solving/perception. The exposure of the adults was similar to the current study and some of the measures were

the same across studies (Fig. 1). Other studies in Egyptian populations comparing adult pesticide applicators to controls have also reported increased neurological, neuromuscular, and psychological symptoms (Amr, 1999), and psychiatric disorders (Amr et al., 1997).

The applicator children show decreased BMI. Organophosphates (OP) exposure has been reported to reduce food intake (Campbell and Hayes, 1974) and may cause these differences. However, an examination of the BMI of both the applicator and control groups shows that it is within the normal range of 18.5–22.5 (WHO, 1995). BMI was included as a covariate in the analysis and was not found to impact performance on the neurobehavioral tests.

Our results also show that there was a significantly lower activity level of AChE in the applicator children compared to the control children. AChE is used as a biomarker for exposure to OP pesticides (von Osten et al., 2004) and the states of Washington and California require cholinesterase monitoring for pesticide applicators. However, because of individual variability, these require a baseline measure and a second measure to indicate a significant depression in cholinesterase activity (Stefanidou et al., 2003). In spite of this recommendation, there are several studies that report only a single cholinesterase level and show a consistent significant association between exposure to pesticides and cholinesterase inhibition in farm workers. Nine studies investigated the effect of pesticide exposure on AChE in pesticide applicators. Three of them measured the AChE before and after exposure and found a significant inhibition in its activity after exposure (Abu Mourad, 2005; Ames et al., 1989; Ohayo-Mitoko et al., 1999). The other studies measured AChE at a single time point and compared it with AChE from a control group. All studies found AChE was significantly lower in the exposed participants than the controls (Chadee and Le Maitre, 1991; Farahat et al., 2003; Misra et al., 1988; Patil et al., 2003; Safi et al., 2005; Srivastava et al., 2000). These findings were replicated in the present study. There was also no significant difference in AChE between the

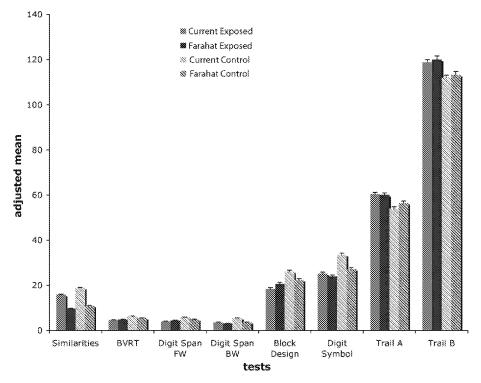


Fig. 1. Neurobehavioral performance of the Older participants in the current study and adult participants from Farahat et al. (2003).

Younger and Older participants either in the applicator or control groups.

The applicator participants also reported more neurological symptoms and scored higher on the personality traits of psychoticism and neuroticism. These findings are similar to other studies with adult workers (Farahat et al., 2003; Kamel and Hoppin, 2004). Two studies in Egyptian populations comparing adult pesticide applicators compared to the controls have reported increased neurological, neuromuscular, and psychological symptoms (Amr, 1999), and psychiatric disorders (Amr et al., 1997).

Applicator children who reported neurological symptoms worked significantly more days than applicator children who do not report these symptoms. They also worked more years than children who do not report these symptoms. These results are similar to studies with adults which found that increased time spent working is associated with increased symptom reporting (Farahat et al., 2003; Kamel et al., 2003; Rohlman et al., 2007).

The association between the level of AChE and neurobehavioral performance, was confirmed by presence of significant correlations between the AChE activity level and the Information, Digit Span and Trail Making tests. These findings address the concerns raised by Roldan-Tapia et al. (2005), about the relationship between NB performance and inhibition of AChE.

There are some limitations of the current study. First, exposure was not limited a specific pesticide. Other pesticides and growth enhancers are applied during the application period including Pyrethroids and Agerin (a biological pesticide). Also, measurement of AChE at only one time point after the spraying season does not provide a measure of the AChE variability of individuals (Stefanidou, 2005). However, these results provide information about a dose–response relationship with duration of exposure and increased impairment in children working as pesticide applicators. They provide a first step as part of larger plan to develop interventions and training to reduce pesticide exposure in applicators.

5. Conclusion

This study showed that children and adolescents who apply pesticides in the cotton fields have significantly lower neurobehavioral performance, report more symptoms and have lower activity levels of serum AChE compared to a control group. The neurobehavioral deficits demonstrated a dose–response relationship between days and years of exposure and NB performance and symptom reporting. Since children around the world are exposed to OP pesticides, these studies suggest the need to evaluate this potential problem is urgent.

Acknowledgments

The study was performed in the Public Health and Community Medicine Department, Faculty of Medicine, Menoufia University, Shebin Elkom, Egypt. We thank the children, adolescents and their parents for their participation in the study. We thank also Dr. Khaled Zyada, the Psychology lecturer, Faculty of Arts, Menoufia University, Egypt, for his help in the selection and administration of the appropriate neurobehavioral tests. We thank also Michael Lasarev, for his help in the statistical analysis.

This manuscript is a part of the MD thesis of Ahmed A. Ismail for his fulfillment of the Doctor Degree in Industrial Medicine and Occupational Health. The entire work was conducted and funded by the Faculty of Medicine, Menoufia University, Egypt. The study was reviewed and accepted by the Medical Ethics committee of the Faculty of Medicine, Menoufia University on July 13 2005.

References

- Abdel-Khalek AM. Development of personality traits. J Contemp Psychol (Egypt) 1994;3:29–54 [in Arabic].
- Abu Mourad T. Adverse impact of insecticides on the health of Palestinian farm workers in Gaza strip: a hematological biomarker study. Int J Occup Environ health 2005;11:144–9.
- Ames RG, Brown SK, Mengle DC, Kahn E, Stratton JW, Jackson RJ. Cholinesterase activity depression among Californian agricultural pesticide applicators. Am J Ind Med 1989:15:143–50.
- Amr MM. Pesticide monitoring and its health problems in Egypt, a third world country. Toxicol Lett 1999;107:1–13.
- Amr MM, Halim ZS, Moussa SS. Psychiatric disorders among Egyptian pesticide applicators and formulators. Environ Res 1997;73:193–9.
- Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity. Neurosci Biobehav Rev 2003;27:3–18.
- Campbell TC, Hayes JR. Role of nutrition in the drug metabolizing system. Pharmacol Res 1974:26:171–97.
- Chadee DD, Le Maitre A. Serum cholinesterase levels of vector control workers in Trinidad West Indies (1979–1987). Ann Trop Med Parasitol 1991;85(3):345–8.
- Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. New York: Academic press; 1988.
- Eckerman DA, Gimenes LS, de Souza RC, Lopes Galvão PR, Sarcinelli PN, Chrisman JR. Age related effects of pesticide exposure on neurobehavioral performance of adolescent farm workers in Brazil. Neurotoxicol Teratol 2007;29:164–75.
- Farahat TM, Abdelrasoul GM, Amr MM, Shebl MM, Farahat FM, Anger WK. Neurobehavioral effects among workers occupationally exposed to organophosphorous pesticides. Occup Environ Med 2003;60(4):279–86.
- Furman J. Cholinesterase monitoring for agricultural pesticide handlers guidelines for health care providers in Washington State. Department of Labor & Industries. WISHA Policy & Technical Services; 2006.
- Handal A, Lozoff B, Breilh J, Harlow S. Neurobehavioral development in children with potential exposure to pesticides. Epidemiology 2007;18(3):312–20.
- Holm S. A simple sequentially rejective Bonferroni test procedure. Scand J Stat 1979;6:65–70.
- Johnson DE, Seidler FJ, Slotkin TA. Early biochemical detection of delayed neurotoxicity resulting from developmental exposure to chloropyrifos. Brain Res Bull 1998;45(2):143-7.
- Kamel F, Hoppin JA. Association of pesticide exposure with neurologic dysfunction and disease. Environ Health Perspect 2004;112:950–8.
- Kamel F, Rowland A, Park L, Anger W, Baird D, Gladen B, et al. Neurobehavioral performance and work experience in Florida farmworkers. Environ Health Perspect 2003;111:1765–72.
- Mansour SA. Pesticide exposure-Egyptian scene. Toxicology 2004;198:91-115.
- Misra UK, Nag D, Khan WA, Ray PK. A study of nerve conduction velocity, late responses and neuromuscular synapse functions in organophosphate workers in India. Arch Toxicol 1988:61:496–500.
- Moser VC, Padilla S. Age- and gender-related differences in the time course of behavioral and biochemical effects produced by oral chlorpyrifos in rats. Toxicol Appl Pharmacol 1998;149(1):107–19.
- Ohayo-Mitoko GJ, Kromhout H, Karumba PN, Boleij JS. Identification of determinants of pesticide exposure among Kenyan agricultural workers using empirical modelling. Ann Occup Hyg 1999;43:519–25.
- Patil JA, Patil AJ, Govindwar SP. Biochemical effects of various pesticides on sprayers of grape gardens. Ind J Clin Biochem 2003;18(2):16–22.
- Rizk LY. The white gold calamity. Al-Ahram Weekly 29 July-4 August 1999;440.
- Rohlman DS, Arcury TA, Quandt SA, Lasarev M, Rothlein J, Travers R, et al. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. Neurotoxicology 2005;26:589–98.
- Rohlman DS, Bailey SR, Anger WA, McCauley L. Assessment of neurobehavioral function with computerized tests in a population of Hispanic adolescents working in agriculture. Environ Res 2001;85:14–24.
- Rohlman DS, Lasarev M, Anger WK, Sherer J, Stupfel J, McCauley L. Neurobehavioral performance of adults and adolescent agricultural workers. Neurotoxicology 2007;28(2):374–80.
- Roldan-Tapia L, Parron T, Sanchez F. Neuropsychological effects of long-term exposure to organophosphate pesticides. Neurotoxicol Teratol 2005;259–66.
- Safi JM, Abu Mourad TA, Yassin MM. Hematological biomarkers in farm workers exposed to organophosphorus pesticides in the Gaza Strip. Arch Environ Occup Health 2005;60(5):235–41.
- Srivastava AK, Gupta BN, Bihari V, Mathur N, Srivastava LP, Pangtey BS, et al. Clinical biochemical and neurobehavioral studies of workers engages in the manufacture of quinalphos. Food Chem Toxicol 2000;38:65–9.
- Stallones L, Beseler C. Pesticide illness, farm practices and neurological symptoms among farm residents in Colorado. Environ Res 2002;90:89–97.
- Stefanidou M, Athanaselis S, Velonakis M, Pappa F, Koutselinis A. Occupational exposure to cholinesterase inhibiting pesticides: a Greek case. Int J Environ Health Res 2003;13:23–9.
- von Osten RJ, Epomex C, Tinoco-Ojanguren R, Soares AM, Guilhermino L. Effect of pesticide exposure on acetylcholinesterase activity in subsistence farmers from Campeche, Mexico. Arch Environ Health 2004;59(8):418–25.
- Weber H. Quick and simple ultra-micro-method for the determination of cholinesterase. Dtsch Med Weschr 1966;91:1927–32.
- WHO. Physical status: the use and interpretation of anthropometry. World Health Organ Tech Rep Ser 1995;854:1-452.